

## Addressing Emerging Pathogens and Parasites in the Galápagos Islands

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### I. Introduction

Six hundred miles off the coast of Ecuador, I sat on the top deck of the ship *La Pinta*, enchanted by the sight of the Milky Way and surrounded by an unfamiliar stillness. A sudden thrash in the water below brought the Galápagos night to life: a needlefish skipped across the waves, stirring up a trail of bioluminescent dinoflagellates in its wake; two seven-foot Galápagos sharks darted after the fish, and a sea lion joined in the chase. In the dead of the night, observing this evening drama unfold, I felt that I was in an untouched environment. Yet “untouched” is far from the reality of the Galápagos archipelago. During the ten days I spent traveling between islands with Professor Durham, fellow Stanford students, and alumni, I encountered invasive fire ants, an introduced rat species, and carcasses of Galápagos sea lions and marine iguanas ravaged by rising ocean temperatures. I saw colonies of blue-footed boobies with abnormally low populations, and I met Galápagos penguins that harbor a *Plasmodium* species known to cause avian malaria.

The Galápagos Islands is no longer an isolated ecosystem: over 25,000 people call the Galápagos Islands home, and 200,000 people visit the national park annually, transported by planes and boats that carry foreign species and pathogens (Galápagos National Park, 2012; Galápagos Conservancy, 2010).

Addressing emerging pathogens and parasites in the Galápagos Islands is an important and complex issue, requiring the sampling of at-risk Galápagos species, planning for disease outbreaks, and designing policy and management practices to mitigate risk of introducing diseases. This problem is especially pressing, as zoologists suspect the 2015–2016 El Niño will compromise the immune systems of endemic Galápagos species, increasing their susceptibility to disease.

Here, I examine the Galápagos penguin as a case study for this potentially imminent threat. I determine that Galápagos penguins are at risk of contracting diseases from domestic chickens, cats, and the introduced *Culex quinquefasciatus* mosquito. The findings lead me to recommend new policy and management practices, including heightened surveillance of disease, strengthened fumigation protocols, and

development of outreach programs to educate locals about transmission of disease in livestock.

## II. The Galápagos penguin: A case study on the impact of introduced diseases in the Galápagos Islands

The Galápagos penguin (*Spheniscus mendiculus*) is endangered due to its large population fluctuations and unusually low genetic diversity (IUCN, 2015). The Galápagos penguin population is currently estimated at only 1,500 (Palmer *et al.*, 2013). Out of 17 penguin species worldwide, the Galápagos penguin is the only species that lives in an equatorial climate (Tui de Roy, 2009). The Galápagos penguin's reliance on the cool waters of the Cromwell current makes this species especially vulnerable to starvation and disease during El Niño conditions. Previous El Niño events have decreased the Galápagos penguin population by as much as 77% (Palmer *et al.*, 2013). It is thought that the harsh El Niño conditions compromise the penguins' immune systems, placing them at higher susceptibility to recently introduced diseases.

Galápagos penguins are especially susceptible to introduced diseases because of population fluctuations and low major histocompatibility complex (MHC) diversity. The MHC is a family of proteins that encode for immune system genes (Parker & Whiteman, 2011). In most organisms, balancing selection acts on MHC, meaning all genes and alleles are selected for equally. The explanation behind this balancing selection is largely contested. One theory is that diversity of MHC genes increases parasite resistance and immunocompetence in the individual (Hedrick, 1998; Parker & Whiteman, 2011). Another possible explanation is negative assortative mating (in which an individual selects a mate with different MHC genes than their own, likely based on odor) (Hedrick, 1998). In the Galápagos penguin, we see a deviation from this trend of high MHC diversity. The Galápagos penguin has been shown to have a reduced average number of differences between alleles (Bollmer *et al.*, 2007; Tsuda *et al.*, 2001; Kikkawa *et al.*, 2005). The average number of differences between alleles is only  $2.0 \pm 1.2$  in the Galápagos penguin, compared to  $9.7 \pm 2.1$  in the Humboldt penguin, the Galápagos penguin's closest relative (Bollmer, Vargas, & Parker, 2007; Kikkawa *et al.*, 2005). Low MHC diversity suggests that there are few differences among the immune system genes in the penguin population. Therefore, an introduced disease could devastate the majority of the Galápagos penguin population.

Low MHC diversity in the Galápagos penguin has been attributed to the founder effect, El Niño events, and inbreeding (Bollmer, Vargas, & Parker, 2007; Parker & Whiteman, 2011). The founder effect refers to the colony of penguins that first arrived on the islands. This initial gene pool was geographically isolated, causing reduced genetic diversity and inbreeding. El Niño events cause population bottlenecks, which result in

the propagation of a very small gene pool during recovery (Palmer *et al.*, 2013). Gene flow between penguin colonies exacerbates the reduced genetic diversity. High migration rates between the different penguin colonies have created a lack of population structure (Nims, Vargas, Merkel, & Parker, 2007). This suggests that the penguin colonies are not genetically distinct from one another. The ramifications of penguin migration mean that a disease causing high mortality in one penguin colony will likely cause high mortality in all other colonies and that penguins can spread diseases throughout the archipelago. Additionally, the Galápagos penguin is immunologically naïve, meaning it has no acquired immunity to viruses such as avian paramyxovirus types 1–3, avian influenza virus, infectious bursal disease (IBD) virus, Marek’s disease virus, avian encephalomyelitis virus, avian adenovirus types 1 and 2, and West Nile virus (Travis *et al.*, 2006).

These findings suggest that the Galápagos penguin population is highly susceptible to disease due to low genetic diversity, population fluctuations, and immunological naivety. This vulnerability suggests a need for a monitoring system to evaluate Galápagos penguin health regularly.

It also suggests the importance of developing a Galápagos penguin captive breeding program, to act preemptively before the population is severely decimated by disease. I will later discuss the details of these proposed initiatives. While I focus here on the Galápagos penguin, it is important to note that low genetic diversity is also an issue in other Galápagos species, such as the Galápagos hawk and land iguana.

### III. Threat of introduced vectors to the Galápagos penguin and other endemic species

Mosquitos carry pathogens that could be transmitted to vulnerable Galápagos populations. The introduction of the *Culex quinquefasciatus* mosquito to the Galápagos Islands demonstrates the ramifications of introducing foreign vectors into susceptible ecosystems. The *Culex quinquefasciatus* mosquito was introduced to the islands in the 1980’s, and is thought to have carried *Plasmodium spp.* (De Roy, 2009). *Plasmodium spp.* is the protozoan responsible for avian malaria, a highly pathogenic disease shown to cause anorexia, vomiting, seizing, and sudden death in birds, including wild penguins.

*Plasmodium spp.* was first observed in Galápagos penguins in 2009 (Levin, Outlaw, Vargas, & Parker, 2009). This was the first reported incident of avian malaria in the Galápagos Islands. Approximately 5% of the 362 penguins sampled had positive PCR results for *Plasmodium spp.*, meaning that *Plasmodium spp.* DNA was detected. Levin, Outlaw, Vargas, & Parker did not observe any clinical symptoms of malaria in the penguins sampled; I will later discuss the implications of this. Phylogenetic analysis

was used to determine that the *Plasmodium* species was closely related to the *P. elongatum* species, which is known to cause severe mortality and morbidity in penguin populations (Levin, Outlaw, Vargas, & Parker, 2009).

The initial report of *Plasmodium spp.* in Galápagos penguins was followed by additional PCR and antibody tests. Palmer *et al.* (2013) sampled 181 penguins at 8 different sites and found anti-*Plasmodium spp.* antibodies in 97.2% of those sampled. The presence of anti-*Plasmodium spp.* antibodies indicates that the majority of penguins had been previously exposed to the parasite. This suggests that the penguins have developed immunity to the *Plasmodium* and that *Plasmodium*-induced mortality is low. Interestingly, there was no gametocyte (mature *Plasmodium* protozoan) detection in the thin blood smears of penguins and PCR results showed that only 9.4% of penguins sampled had an active parasite infection (Palmer *et al.*, 2013). These findings raise a number of questions regarding the absence of gametocytes and absence of disease symptoms. Dr. Patricia Parker, a leading expert on Galápagos penguin malaria, theorizes that the *Plasmodium* is not fully maturing in the penguin and thus does not develop gametocytes, the mature protozoans. This means that the penguin is not a competent host for the *Plasmodium*, which suggests that a different avian species in the Galápagos is the host of *Plasmodium*. Additional research is needed to determine the host and elucidate the mechanism of *Plasmodium* transmission.

Another unsolved mystery is the absence of avian malaria symptoms in the penguins sampled. It is possible that researchers are only seeing the surviving penguins, and that many are actually dying. Galápagos naturalist guides have reported groups of dead penguins (P. Parker, personal communication, 2015). Unfortunately, a law prohibiting naturalists from collecting dead specimens has prevented scientists from examining these penguins to determine a cause of illness. The second possibility is that the penguins can tolerate the *Plasmodium* species during benign environmental conditions, which occurred in 2003–2009 when the samples were taken (P. Parker, personal communication, 2015). The physiological stress caused by the 2015–2016 El Niño may trigger a more severe response to the *Plasmodium*, causing illness and even death. Research in 2016 may shed light on the effects of environmental conditions on the pathogenicity of the *Plasmodium*.

#### IV. Transmission of disease between domestic species and Galápagos endemics

Another chief area of concern is the spread of diseases transmitted by domestic animals of the Galápagos, specifically chickens and cats. Cats in the Galápagos Islands have been shown to harbor *Toxoplasma gondii*, a protozoan parasite that causes diarrhea, respiratory distress, and death in

avian species. Levy *et al.* (2008) sampled 52 cats on Isabella Island and found that 62% of the cats had antibodies to *T. gondii*. This is significant because cats and penguins occupy the same geographic area in the Galápagos. Deem *et al.* (2010) sampled 298 Galápagos penguins on Isabella Island and Fernandina Island and found a total *T. gondii* antibody prevalence of 2.3%. Interestingly, *T. gondii* antibody prevalence was significantly higher on Fernandina, an island without cats, than on Isabella, an island with cats ( $p = .02$ , Fisher's exact test). There are a few theories explaining this data. It is possible that *Toxoplasma* oocysts were dispersed by currents to Fernandina. In fact, it has been shown that *Toxoplasma* oocysts can survive and sporulate in seawater for months (Lindsay, David, & Dubey *et al.*, 2003). It is also possible that there is an intermediate host transmitting *T. gondii*. As previously discussed, penguins move throughout the archipelago and may have brought the protozoa from Isabella to Fernandina.

The pathogenicity of *T. gondii* in Galápagos penguins is currently unknown, and Deem *et al.* (2010) did not report any symptoms of illness in the penguins found positive for *T. gondii*. It is possible that environmental changes, co-infection with other parasites, or other stressors may cause symptoms to develop, analogous to the current avian malaria situation. Though more research on the pathogenicity and fitness costs of *T. gondii* is needed, this evidence suggests that Galápagos penguins are at risk of contracting diseases from cats.

Evaluation of chickens from farms on San Cristobal and Santa Cruz Islands demonstrates that the chickens harbor diseases that threaten penguins. Gottdenker *et al.* (2005) sampled 100 chickens from San Cristobal and Santa Cruz Islands and found a variety of bacterial, viral, and parasitic diseases, including avian adenovirus I, Marek's disease, Newcastle disease, and infectious bursal disease (IBD). Of greatest concern for the Galápagos penguin population is the presence of Newcastle disease in 12% of chickens sampled. Newcastle disease causes high mortality and morbidity in avian species (Gottdenker *et al.*, 2005). Penguins are known to be especially susceptible to Newcastle disease (Docherty & Friend, 1999). It follows that this highly pathogenic virus could pose a significant threat to the Galápagos penguin and other endemic avian species.

#### V. Recommendations for policy and management practices to address threat of introduced diseases

In addressing the threat of introduced diseases, we need to examine current policy and management practices and evaluate their efficacy in the context of environmental changes and the vulnerabilities of Galápagos species. Current policy to curb introduction of new diseases into the Galápagos focuses on fumigation. The Inspection and Quarantine System

for Galápagos (SICGAL) and the Ecuadorian national agency AGROCALIDAD are responsible for inspecting ships and aircraft entering the Galápagos Islands (Parque Nacional Galápagos, 2015). All planes must be fumigated upon arrival at San Cristobal or Baltra airport and luggage is scanned for any live specimens. Private (foreign and domestic) vessels are required to have a certificate of fumigation from the last port visited prior to arriving in the Galápagos. There is, however, no system in place to prevent the transport of organisms between the islands (De Roy, 2009).

It is critical that we minimize opportunities for introducing diseases into the Galápagos ecosystem. A study conducted in 2009 assessed 126 airplanes at Baltra and San Cristobal airports and found 8 *Culex quinquefasciatus* mosquitos and 74 live invertebrates (Bataille *et al.*, 2009). *Culex quinquefasciatus* mosquitos carry a number of diseases that have yet to be introduced to the archipelago, such as West Nile virus, which could devastate Galápagos ecosystems. In order to better manage the threat of introducing diseases, SICGAL (the inspection and quarantine system) needs sufficient funding to implement and enforce fumigation policies. SICGAL's funding, however, has been recently decreased by 20% despite the rise in transportation in and out of the Galápagos (Parque Nacional Galápagos, 2015). Moreover, enforcement of the fumigation protocol for vessels has been challenging (Bataille *et al.*, 2009), and there is no policy to decrease risk of transporting mosquitos or other species between the islands. A potential policy could involve using an environmentally safe disinfectant to clean clothes and equipment, or providing educational outreach to Galápagueños and researchers to raise awareness of the importance of washing shoes and supplies when travelling between islands.

Conservation of the Galápagos penguin should involve a strong baseline-monitoring program to allow for early detection of disease. Because the penguin is at risk of contracting highly pathogenic diseases with sudden onset of death, it is imperative that diseases are detected early. Dr. Patricia Parker of UMSL, in collaboration with the St. Louis Zoo, the Charles Darwin Foundation, and the Galápagos National Park, has recently implemented an avian disease surveillance system. I recommend that the Galápagos National Park change its policy on collecting specimens to allow Galápagos naturalists to collect dead organisms in pre-approved circumstances. This would provide more timely data in the event of a disease outbreak and would allow for a stronger surveillance system. Such a program would need to be closely monitored to avoid potential risks of removing species from the environment. To accomplish this, the Galápagos National Park would pre-approve the request of the researcher for specimens, and a strict specimen quota would be established, determined by the nature of the project. Additionally, naturalists would need to be trained in proper specimen

collection and storage protocols.

It may also make sense to establish a captive breeding program for the Galápagos penguin because introduced diseases severely threaten its low and fluctuating population. However, the creation of a captive breeding program would require extensive research on Galápagos penguin behavior and habitat. Consequently, the expenses and time necessary to create a captive breeding program could make it economically infeasible under current conditions.

To address the threat of diseases transmitted by domestic animals, we should reduce the number of cats to the extent possible by sterilization. Free or low cost spay and neuter clinics in towns such as Puerto Ayora, Puerto Villamil, and Puerto Baquerizo Moreno would help decrease the cat population. The population of domestic chickens cannot be controlled in the same manner, however, because chickens are integral to Galápagos agriculture and serve as an important food source. It is therefore imperative that we research ways to improve the health of domestic chickens and to contain disease outbreaks. One potential initiative is educational outreach programs to teach locals about the effects of lighting, ventilation, and sanitation on chicken health. The program could also teach people to recognize signs of illness in chickens, so that ill chickens can be isolated from others as soon as possible to avoid transmission of disease. Another approach is vector control, as avian diseases are often vector-borne. Vector control could involve educating people about practices to reduce mosquito populations around farms, such as removing standing water when possible. Using pesticides to kill vectors would not be an effective approach, however, because it would be difficult to target the introduced species (*Culex quinquefasciatus*) without targeting native mosquito species.

## VI. Conclusion

Introduced pathogens and parasites pose a threat to endemic Galápagos species, and these species are especially vulnerable due to low genetic diversity and changing environmental conditions. Interestingly, introduced pathogens and parasites—arguably the greatest threat to this fragile ecosystem—are largely invisible to the eye in the Galápagos. Like many visitors to the Galápagos, I was challenged to look past my first impression of these intrepid characters; it is easy to see the vibrant blue of the flightless cormorant's eye, observe a penguin diving into the sea, or witness the mating dance of the blue-footed booby—and assume that these unique species are thriving and unchallenged. Yet, hidden underneath their dazzling adaptations and charismatic fearlessness, lies the threat of pathogens. As global climate change increases the frequency and severity of El Niño events, pathogens and parasites threaten to decimate endemic populations. It is imperative that we address these concerns proactively, despite their hidden nature. Future work on the epidemiology of

introduced diseases, as well as development of policy and management practices, will reveal new strategies for conservation in the face of these emerging threats.



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